

BIOCONTROL OF PHYTOPATHOGEN USING LACTIC ACID BACTERIA FROM FERMENTED FOOD AND APPLICATION IN SUSTAINABLE AGRICULTURE

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ABSTRACT :

*Probiotics are living microorganisms belonging to mainly lactic acid bacteria. They have many beneficial properties in human health as well as in agriculture also. The present work idea is focused on the isolation and identification of novel probiotic microbes from ethnic fermented foods of indigenous tribes of north-east region. A variety of region specific fermented foods and beverages such as Kharoli, Gundruk, Goimoz, Jim tenga, Lung-seij, Miya mecheng, Ekung, Muya awandru, and Nichaow are traditionally produced and consumed, and even locally marketed in North-East India. These fermented foods are consortia of beneficial lactic acid bacteria and their antimicrobial activity and other properties have applications as anti-phytopathogen, biopesticide, biofertilizer, phytostimulant etc.. Probiotic microorganisms confer direct benefits to the plant acting as biocontrol agents for their antagonistic activity against phytopathogens *Xanthomona scampetris*, *Erwinia carotovora*, and *Pseudomonas syringae*. Probiotic bacteria can act as phytostimulant by can enhancing phyto hormones like auxin, cytokine, gibberelic acid and ethylene. These bacteria has potential in seed germination. *Lactobacillus* has potential impact in seed germination of Bengal gram (*Cicer arietinum*), Peas (*Pisum sativum*), Mustered seeds (*Bassica hirta*), rice (*Oryza sativa* L). The present work idea will explore novel probiotic strains for potent applications in agriculture.*

KEYWORDS: Probiotics, Fermented Foods, Antiphytopathogen, Biocontrol Agent & Phytostimulant

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INTRODUCTION

The world's ever-increasing human population leads to more requirements of food but various pests and plant diseases cause loss of crop yield, deteriorated quality and contamination of produce with toxic agrochemicals. To combat the situation, farmers are intensively applying several synthetic agrochemicals to control pests and phytopathogens in agriculture. Indiscriminate use of several agrochemicals like fungicide, pesticide etc. has emerged several hazards like developing resistance in phytopathogens towards the applied fungicides, alteration in physiological, photosynthetic and other metabolic activities in the plant reducing growth rate and crop production. Fungicide toxicity is not always restricted to the target pathogenic organism, it also ruins the other beneficial microbes. Besides, agrochemicals adsorbed in soil are likely to persist for longer periods of time due to their decreased availability for microbial degradation. The pesticide and fungicide residues in the soil also contaminate ground water and adversely affect aquaculture and ecosystem. Through fungicide residues in crops, these harmful chemicals enter into food chain and cause health hazards in humans. Fungicide residues in cattle feed enters into cattle and finally goes to milk and other food chain and affect human health.

Besides, another concern of huge use of P containing chemical fertilizer to overcome limited productivity due to P deficiency in soil because of unavailability from complex phosphate salt from soil by plants. Thus abusive use and adverse effects of these agrochemicals has diverted public concern towards developing ecofriendly, environment benign alternative method of plant protection and nutrition by biocontrol approach for remediation of poor soil condition for sustainable agriculture. As per available literature, several microbes like *Bacillus sp.*, *Pseudomonas sp.*, Lactic acid bacteria etc. have been found to produce a variety of antimicrobial compounds like bacteriocin, siderophores, hydrolytic enzymes, secondary metabolites and volatile organic compounds. These compounds have been found to suppress growth of different phytopathogens and can be effective biocontrol agents. Lactic acid bacteria have been proved to be a potent phytostimulating and phosphate solubilizing agent also. Northeast region of India is a rich source of biodiversity and inhabited by indigenous tribes. These aboriginal inhabitants have been practicing the preparation of different fermented foods from vegetables, local herbs, bamboo shoot, fish and beverages from rice. These ethnic fermented food and beverages of north east India are rich source of different lactic acid bacteria (LAB). All these indigenous recipes are yet to be explored for potent anti-phytopathogenic and phosphate solubilizing strains. Therefore, the objective of the current work proposal is to isolate novel microbial strain(s) from these ethnic fermented food, exploring their anti-phytopathogenic, phosphate solubilizing and phytostimulation property for applying as effective bio control and phytostimulation agent.

Novelty

Extensive use of agrochemicals causes different hazards to the environment and threats to human health. Therefore human preference for chemical residue-free produce has increased demand for biocontrol agents for plant protection management. Since there is not enough availability of target specific or broad spectrum biofungicides still today, huge scope lies to screen new microbial strain(s) and exploring their unusual anti phytopathogenic, phosphate solubilizing and phytostimulating activity. The traditional fermented foods and beverages of Northeast region (NER) have not been widely explored yet for anti phytopathogenic microbial species. As per peer-reviewed literature, Lactic acid bacteria, *Bacillus sp.*, *Pseudomonas sp.* have been reported to show anti-phytopathogenic activity and be present in different Asian fermented foods. Therefore these ethnic fermented foods of NER may also be a rich source of potent anti-phytopathogenic microbial species. Hence the proposed work aims to screen potent strains based on anti-phytopathogenic activity and to explore desirable properties like phosphate solubilization, phytostimulation, wide viability range of temperature, pH, humidity, similar activity in invivo trials, improvement in antioxidant of produce in hydroponic system. The outcome of the proposed study may explore a novel anti phytopathogenic microbial strain(s) for potent application as biofungicide.

Rationale

Over the past decades, extensive use of agrochemicals has improved crop quantity and quality as well but simultaneously emerged hazards of environmental pollution, adverse effect on human health, ecosystem etc. Therefore human attention now has been diverted towards exploring biocontrol agents in agriculture. As per literature survey, Lactic acid bacteria, *Bacillus sp.*, *Pseudomonas sp.* etc. have been found to produce several antimicrobial compounds like bacteriocin, siderophores, hydrolytic enzymes, secondary metabolites and volatile organic compounds which can inhibit phytopathogenic bacteria and fungi. Fermented food and beverages are usually rich sources of potent lactic acid bacteria while *Bacillus sp.* has been found to be present especially in soya based fermented food. As per literature, it has been reported, bamboo shoot fermented food, sauerkraut, fermented olive from north east region of India has *Pseudomonas sp* and *Pseudomonas fluorescens*. (Tamang et. al 2016). Lactic acid bacteria has been found to show anti-phytopathogenic

activity against *Fusarium oxysporum*, a fungi affecting capsicum plant. *Lactobacillus sp.* has the ability to neutralize the toxic effect of *Fusarium oxysporum* from capsicum plant (Hamed et al 2011). *Colletotrichum capsici*, *Fusarium oxysporum* and *Pythium ultimum* (Mabrok et al 2012) are causative agents of fungal infection of chilli, tomato and cucumber plants and lactic acid bacteria has anti fungal activity against these phyto pathogens. (Lutz et al 2012). *Penicillium*, *Botrytis cinerea*, *Monilinia laxa* and other fungi produce mycotoxins. Mycotoxins are secondary metabolites produced by various fungus can spoil fresh fruits and vegetables. (Rosalia et al. 2008, Spadaro et al. 2004). Fresh fruits and vegetables contain various lactic acid bacteria which has anti phytopathogenic activity against various phyto pathogen like, *Xanthomonas campestris*, *Erwinia carotovora*, *Penicillium expansum*, *Monilinia laxa*, *Botrytis cinerea* etc. Phenylalanine ammonia-lyase, guaiacol peroxidases, polyphenol oxidase, beta-1,3-glucanase etc are defence enzymes. Bacteriocin or bacteriocin like substances, produced by lactic acid bacteria, can inhibit phytopathogenic fungus. It has been reported, phosphate solubilizing, nitrogen fixing and auxin production activity is present in lactic acid bacteria. (Uma et al. 2018). LAB are probiotic in nature. These bacteria produce bacteriocins and have a lot of fascinating properties as suitable biopreservant and are regarded as safe and non-toxic on eukaryotic cells. *Bacillus sp.* having several antagonistic activity like producing lipopeptides based secondary metabolite, enzymes have been found highly active against fungal plant pathogens (Hashem et al. 2019). Apart from antagonistic behavior, *Bacillus sp.* have phyto stimulating property as it promotes biosynthesis of plants growth hormones gibberellic acid and IAA (Hashem et al. 2019). *Pseudomonas fluorescens* has anti-phyto pathogenic activity. This is abundant in natural soils and plant root systems. They can use many plant discharged materials as a nutrients. Fluorescent *Pseudomonas* has various important traits in bacterial fitness. These microorganisms can adhere to soil particles, rhizoplane. They can synthesis antibiotics, produce hydrolytic enzymes. Moreover, they have motility and proteolytic. This bacteria has the ability to fix nitrogen, solubilize phosphate, chelation of iron and production of phyto hormone. These all traits are plant growth promoting factors. (Panpatte et al. 2016). As per literature reports, their (LAB, *Bacillus sp.*, *Pseudomonas sp.*) presence in different fermented foods and respective antimicrobial and phytopathogenic activity have actually urged the current proposal to focus on screening potent microbial species from indigenous fermented foods of North east region (NER) for its enriched biodiversity. Capsicum is an economically important vegetable. Capsicum produces various bio chemical compounds which have health benefit. Capsicum has volatile oils, carotenoids, fatty acids, vitamins, minerals etc. The current research will show anti phytopathogenic and biostimulant activity of lactic acid bacteria and phosphate solubilizing bacteria against capsicum fungal disease. It increases oxidation by which calories are decreased. Capsicum helps reduce pain by relieving endorphins. Vitamin A and C present in red and green capsicum. Since ethnic foods and beverage of NER have not been widely explored yet for anti-phytopathogenic, phosphate solubilizing and phyto stimulating traits, and there is chance of exploring rare/novel strains with immense potential from these traditional recipes, the present work proposal instigates to investigate anti- phytopathogenic, phosphate solubilizing and phyto stimulating potential of isolated microbial species from traditional fermented foods and beverage of NER. Several fermented foods, made from vegetables, bamboo shoot, fish and fermented rice based beverage will be collected from seven states of NER for study. After exploring effective anti-phytopathogenic, phosphate solubilizing and phyto stimulating activity, the study will be carried on in seed germination, pot study and hydroponic system to evaluate their relevant potential for prospective application as biocontrol and phyto stimulation agents.

National Importance and Social Relevance

Extensive use of agrochemicals in conventional farming causes increased resistance in phytopathogens, alteration in plant photosynthetic activity, reduced and inferior crop production. The adverse effects are also found in killing beneficial microbes of soil, poor soil condition, contaminating ground water, destroying aquaculture and polluting environment. Affecting biodiversity totally destroy rural life especially for the below poverty line who are used to collecting from nature, aquasystem for their livelihood. Agrochemical residues in produce and through food chain has been severe threat to human health and animals. Therefore in the recent past, organic farming with biological plant protection has been the best option to way out of these hazards. But farmers are not much interested to adopt biofungicides instead of conventional agrochemicals because potential benefits with organic farming have not yet been very visible. Besides, variety of target specific and broad spectrum biofungicides are not available/explored yet and application of biocontrol requires skilled plant protection management. Though farmers get better price for residue free organic produce, organic farming requires more labor and investment in biocontrol agents. Since biocontrol beneficiates farmers by replenishing poor soil conditions, beneficial microbes, crop's resistance to climate change and eradicates all environmental hazards in long run by sustainable agriculture, stern steps are to be initiated by the government to encourage farmers to adopt this. The government will have to address less pollution issues of environment, water and national health issues in long run. Government can encourage farmers by spreading awareness programme, subsidized small scale biofarming projects to prove better revenue and replenishment of ecosystem, employing food safety regulations for minimizing agrochemical residue in produce etc.. The current proposal aims to isolate potent anti-phytopathogenic microbial strain(s) for potential application as biofungicide. Hence the outcome of the proposed project is supposed to contribute to the national biocontrol initiative for plant protection.

NATIONAL & INTERNATIONAL STATUS

International Status

Lactobacillus is a well known probiotic bacterium. Probiotic lactic acid bacteria secrete some proteins which has probiotic effect. Probiotic proteins accumulated when bacteria is growing inside the culture media. These probiotic proteins secreted by LAB has the ability to intercommunicate molecular level between bacteria. They can also monitor bacterial environments (Van Pijkeren *et. al.* 2006). It has been reported, these probiotic proteins of LAB play an important role in cross-talk with the plant host. These probiotic lactic acid bacteria strains act as biocontrol agents and they have immunomodulation property also (Borja Sanchez *et. al.* 2008). Lactic acid bacteria (LAB), specially *Lactobacillus*, are gram positive, anaerobic, non-sporulating and acid tolerant bacteria. These bacteria have antifungal activity also. (Jeong-Dong Kim 2005). Many LAB strains have been isolated from fermented foods, fresh fruits and vegetables, but very limited studies were done on antifungal potential of LAB isolated from agricultural soils (Canpolat *et. al.* 2018, Fakri *et. al.* 2018). *Lactococcus lactis* has ability exhibited antifungal activities against *C. capsici* with minimum inhibition concentrations. LAB is a novel promising bacterial group in the environment which has ability to control soil born pathogen. LAB can be used as biocontrol agents against some plant fungal infections. It has been reported that post-harvest disease of fruits and vegetables can be controlled by certain probiotic strains of lactic acid bacteria. But there is no evidence that these strains can protect plant root from soil-born fungus (Lutz *et. al.* 2012). 294 isolates of probiotic strains have been isolated from compost of two origins and the rhizosphere of maize, rye, carrots, garden soils and shows anti phytopathogenic activity against *Pythium sp.* (Lutz *et. al.* 2012). 75% of isolated strain shows a prohibitory effect. Pot experiment had been done in infected *Capsicum annuum* plant with the best isolates. The toxicity of pathogen is being

nullified by isolated lactic acid bacteria and it can protect the plant from *Pythium sp.* Capsicum seeds were treated by lactic acid bacteria and the percentage of seed germination was improved.

Previous reports stated that only *Lactobacillus planterum* has the ability to kill and nullify the toxicity exerted by various kinds of plant pathogens viz. *Penicillium expansum*, responsible for blue mold disease in apple and *Xanthomonas campestris* for black rot disease in vegetables (Trias et al. 2008). Plant-fungal pathogen, *Fusarium oxysporum* cause fusarium wilt disease in capsicum plant. This fungus halts the plant growth of capsicum plant. Lactic acid bacteria was tested against this phyto pathogen. (Hamed et al. 2014). Direct treatment of capsicum seeds with lactic acid bacteria broth culture can determine the effect of root-rot disease. This treatment of LAB broth promote plant growth which are infected by *Fusarium oxysporum*. It has been reported that lactic acid bacteria is an excellent anti phyto pathogenic strain. A study has been conducted on *Lactobacillus planterum* LAB C5 and LAB G7 to protect the capsicum plant against some fungal pathogen Further, improvements in seed germination, shoot- and root-length as well as in improving plant biomass were observed in the LBA treated plants (Asma et al. 2013, Limanska et al. 2013, Nataliya et al. 2013, Azziz et al. 2014, Bellishree et al. 2014, Damodarana et al. 2014, Kang et al. 2014). *Colletotrichum capsici*are ubiquitous fungal pathogens that cause anthracnose disease of chilli plant. Although chemical fungicide can be used to manage the disease, but excessive use of the fungicide can lead to development of fungicide resistant as well as human health concern. There are limited reports on lactic acid bacteria can act as anti phyto pathogen. Some strains of lactic acid bacteria can be used as biocontrol agents against soil-born pathogens.

Hydroponic system is a soilless agriculture system, where plants are grown with mineral nutrient solution. In hydroponics, roots are submerged into nutrient rich solution. Availability of land for cultivation is a serious threat now a day. With the help of hydroponic system, this problem can be solved. Plants grow faster, though it's roots are smaller. Plants are kept closer in growing condition. The main difference between soli-based agriculture and hydroponic system is it needs smaller space and 1/20th of total water. The chances of disease, soil-borne insect pest and weed infection is very little here. Hydroponic system provides effective nutrient regulation, planting density will be higher. By using this system yield is increased per acre and production of food quality is better. (Sardare et.al. 2013). Capsicum is high value vegetable. Cultivation of capsicum is high-risk activity because different pests and fungus infect capsicum. The price is also fluctuate in the market and it need great requirement of inputs and services. To maintain the quality of vegetables high cost is required. Quality of vegetables and crops should be better and for this new cultivation and management technique should be adopted. Hydroponic system is a platform where high planting densities can be achieved. The risk of pests attack and disease is reduced when crop cycles slows down. Finally, with the help of hydroponic system planting density is high, yields are good, size of fruits and vegetables are large and quality is uniform. (Cardoso et. al. 2018). It has been reported that vegetables and fruits cultivated in hydroponically increase antioxidant in fruits and vegetables (Ding et. al. 2018).

National Status

Dairy products contain consortia of microorganisms, in which lactic acid bacteria are one of the principle microorganisms. Lactic acid bacteria (LAB) acid producing bacteria which are used in food industry to prepare starter culture for dairy products. LAB are one of the most powerful prokaryotes which produce lactic acid to sour milk. These bacteria are diversified in their species, they can be cocci or rod shaped. They contain typical metabolic and physiological features. Fermentation of carbohydrate produce lactic acid which is the major end product of LAB. The use of lactic acid bacteria along with agriculturally important microorganisms like *Trichoderma*, *Rhizobium*, *Pseudomonas* etc., are practiced to

harness the combined effect of both the organisms for effective crop production (**Uma et. al. 2018**). Plant surface and plant products are a rich source of lactic acid bacteria. These LAB can be used as biocontrol agents against phytopathogens *Xanthomonas campestris*, *Fusarium oxysporium* f. sp. *capsici*, *Pseudomonas syringae*, *Pythium* sp. (**Visser et. al. 1986**).

Capsicum annum is an important spicy vegetable, cultivated through the world. It has different medicinal properties in it. *Capsicum* is a flowering plant which is similar to solanaceae family. *Capsicum* genus consists of 20-25 species. *Capsicum annum*, *Capsicum baccatum*, *Capsicum chinense*, *Capsicum frutescens* are main species which are cultivated. Chili produces various bio chemical compounds which have health benefit. Chili has volatile oils, carotenoids, fatty oils, vitamins, mineral etc. Chili increase oxidation by which calories are decreased. Chili helps reducing pain by relieving endorphins. Vitamin A and C present in red and green chili. In India, chili is one of the most important materialistic vegetables. India is the world's 4th largest country to produce chili. But in India, the average productivity of chili is 1/10 (ton/hect), whereas China, Taiwan and Mexico produces 3/10 (ton/hect). The main reason for this low productivity of chili in India is biotic and abiotic stress which basically leads to different diseases in chili plant. Anthracnose is one of the major diseases in chili, caused by *Colletotrichum* sp. It reduces quality and yield of production of chili. (**Lakshmi et. al. 2014, Kaur et. al. 2010**). Frog eye leaf spot is another disease caused by *Cercospora capsici* fungal infection. Leaves, petioles, stems, are affected by this fungus. The symptoms of this disease is appearance of small, circular, oblong chlorotic lesions. After some time these lesions turned into necrotic with a sporulating light gray centre and blue gray margin. Concentric rings may be observed as individual lesions expand. These lesions look like frog-eye. Thus the name of the disease becomes frog eye disease. There has been reported that *Pythium* spp., *Rhizoctonia solani* and *Fusarium* spp. are causative agents for damping off or root rot disease of *Capsicum annum*. Young seeds become soft due to decaying of tissue in the collar region. *Fusarium oxysporium* f. sp. *capsici* cause wilt disease in upper portion of leaves and lower stem and roots in capsicum plants. There has been reported that *Pseudomonas fluorescens* treated capsicum seeds has antagonistic effect on these phytopathogens (**Abhishek et. al. 2014**). Chemical treatment has negative effect on plants as well as human health and environment also. Lactic acid bacteria are probiotic microorganisms and they are safe for human as well as animal consumption. LAB produce anti phyto pathogenic substances which are acetic acid, lactic acid, hydrogen peroxide, several bacteriocins. Lactic acid bacteria also secrete several types of anti fungal compounds like cyclic di-peptide, phenyllactic acid cyclo (Phe-Pro) compound. (**Tomas et. al. 2004**).

Culture of LAB and their supernatants has anti phyto pathogenic activity against phytopathogens like *Colletotrichum capsici*, *Fusarium oxysporum* and *Pythium ultimum* respectively which cause disease in Chilli, Tomato and Cucumber. (**Vasanthakumari et. al. 2014**). Fresh fruits, vegetables, soil, and milk products are rich source of LAB which is safe for human and animal consumption. It has been reported that *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus buchneri*, *Leuconostoc* spp., and *Weissella cibaria* isolated from fresh fruits and vegetables showed in vitro antagonistic activity in overlay assay. (**Ojekunle et. al. 2017**). Organic acids, hydrogen peroxidase and siderophores, produced by LAB has anti phyto pathogenic activity against fungal pathogen. (**Said et. al. 2019**). Phyto pathogenic activity of spoilage bacteria and fungi namely *Xanthomonas campestris*, *Erwinia carotovora*, *Monilinia laxa* and *Botrytis cinerea* on apple can be stopped by lactic acid bacteria isolated from fresh fruits and vegetables. (**Trias et. al. 2008**). LAB can repress soil-borne disease and can enhance plant growth promoting factors. (**Lutz et. al. 2012**). Lactic acid bacteria can nullify the toxic effect of phyto pathogenic fungi of post-harvested fruits and vegetables. (**Konappa et. al. 2016**). There are

various properties present in lactic acid bacteria which makes them interesting microorganisms to control phytopathogens. (Konappa et. al. 2016). The technology for mass production of LAB is readily available in the food industry.

Innovation

Several plant diseases are causing severe loss of crop production, quality deterioration and farmers are using agrochemicals to combat the situation yielding contaminated produce with agrochemical residues. Conventional farming with extensive agrochemicals is also causing resistance in phytopathogens to chemical fungicides, environmental pollution and health hazards. Therefore human preference for organic produce creates huge demand for biological plant protecting agents to use in organic farming. The proposed work aims to isolate a novel anti-phytopathogenic and phosphate solubilizing microbial strain(s) which can be formulated into an effective biofungicide for applying in organic farming. The potent strain(s) having plant disease control and increased crop yield potential will experience higher demand than conventional agrochemical for applying in sustainable agriculture. The potent strain(s)/high value product will satisfy the existing need for new biofungicides while ensuring food security of the produce. In the proposed study, capsicum will be taken as model plant since it is one of the most popular and economically important vegetable crops. And the isolated strain(s), especially been effective to inhibit capsicum plant phytopathogens, will provide better option for farming organic produce and processed products.

Novelty

Extensive use of agrochemicals causes different hazards to environment and threat to human health. Therefore human preference for chemical residue free produce has increased demand for biocontrol agents for plant protection management. Since there is not enough availability of target specific or broad spectrum biofungicides still today, huge scope lies to screen new microbial strain(s) and exploring their unusual anti phytopathogenic, phosphate solubilizing and phytostimulating activity. The traditional fermented foods and beverages of Northeast region (NER) have not been widely explored yet for anti phytopathogenic microbial species. As per peer reviewed literature, Lactic acid bacteria, *Bacillus sp.*, *Pseudomonas sp.* have been reported to show anti-phytopathogenic activity and be present in different Asian fermented foods. Therefore these ethnic fermented foods of NER may also be a rich source of potent anti-phytopathogenic microbial species. Hence the proposed work aims to screen potent strains based on anti-phytopathogenic activity and to explore desirable properties like phosphate solubilization, phytostimulation, wide viability range of temperature, pH, humidity, similar activity in invivo trials, improvement in antioxidant of produce in hydroponic system. The outcome of the proposed study may explore a novel anti phytopathogenic microbial strain(s) for potent application as biofungicide.

Scope of Industrial Application

The proposed study will screen ethnic fermented foods and beverages of North East region to isolate a novel strain(s) which is/are expected to have potent anti-phytopathogenic, phosphate solubilizing and phytostimulating property. Since a number of severe hazards of using agrochemicals have diverted human attention towards the application of biocontrol agents for plant protection in agriculture, a novel strain(s) will have immense potential in formulating a biofungicide for specific phytopathogen target attack. As encouraging organic farming has been a national mission, novel potent anti-phytopathogenic isolate(s) can provide different products like biofungicide seed treatment formulation, microbial biofungicide technology. Besides phosphate solubilizing activity of the novel strain(s) will help to reduce use of P

containing chemical fertilizer also. Therefore the findings of the current work proposal seem to have immense potential for industrial application.

Commercialization Potential

Severe hazards of using agrochemicals on the environment, human health and animals has diverted human preference for organic and residue-free food. This has influenced farmers to grow traditional seeds with biological crop protection products like fungicide. As a consequence, demand for new biofungicides has increased tremendously for sustainable farming. In this regard, the current research proposal aims to screen traditional fermented foods and beverages of North East region to isolate a novel strain(s) based on the following attributes:

- Potent anti-phytopathogenic property to be effective biofungicide
- Phosphate solubilizing activity to improve bioavailability of soil phosphate to plants and reducing chemical P fertilizer
- Phytostimulating property for increased crop yield
- Viability in broad range of temperature, pH, humidity
- Similar activity in in-vivo, pot and field study
- If broad spectrum, then for several plant disease control, : if narrow spectrum, then for target phytopathogen killing

Therefore the isolated novel strain(s) with the above properties is supposed to have immense commercialization potential as biological control agent in agriculture and can be developed in to a biofungicide formulation.

Business Strategy

The proposed research work will extensively focus on screening a potent anti-phytopathogenic strain(s) to be a potent biofungicide. All relevant research work like characterization, in-vitro and in-vivo study, phosphate solubilizing, phytostimulating activity etc. to be carried on. Extended research work to be carried on in field trials and developing biofungicide seed treatment formulation and biofungicide technology for field application.

- Developing a cost effective, easy-to-use product/technology so that farmers from developing countries like India can afford and apply.
- Application for registration, getting approval, issuing patents.
- Tie up with a Government agency/cooperative/ company for production and contacting regional distributors.
- Advertisement/media activities about new products before product promote/launch.
- Government initiative to arrange/increase awareness program about beneficial effect of biofungicide.
- Getting feedback/market survey from farmers, collecting information/buy similar product from market to compare with own product for improvement/ correction etc.
- Looking in to the strategies of existing and new entrants competitors / companies, realistic evaluation of own status and to be in competition with low cost and effective product.

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